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Reutilization of carbon sources through sugar recovery from waste rice straw

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ABSTRACT

Rice straw was utilized for the cultivation of *Phanerochaete chrysoporium* to produce cellobiose dehydrogenase. The composition of the rice straw after fermentation was found to be 28.77% glucan, 19.05% xylan and 54.81% other lignin containing sugars. The glucan and xylan content decreased due to the consumption of glucan and xylan by *P. chrysoporium*. After fermentation, the rice straw waste was subjected to chemical pretreatment to remove lignin. The effect of dilute acid pretreatment was not notable because of the glucose loss. However, when the rice straw after fermentation was treated with aqueous ammonia, the composition changed to 44.73% glucan, 25.43% xylan and 29.52% other lignin containing sugars. The aqueous ammonia pretreatment was optimized and an ammonia concentration, reaction time and temperature of 20%, 6 h and 60 °C, respectively, were determined to be the optimal pretreatment conditions After removal of lignin, the initial reaction rate was increased to 0.009583 g/L s, which was about 3 fold higher than the rice straw after fermentation. X-ray diffractometry was performed to investigate the crystallinity index, and the XRD results showed that biological treatment and the combination of both biological treatment and chemical pretreatment decreased the crystallinity index.

1. Introduction

Cellobiose dehydrogenase (CDH) is an extracellular enzyme that is produced under cellulolytic conditions by a number of wood degrading fungi. Although the biological function of CDH is still not fully understood, it has been shown to degrade cellobiose to glucose and oxidize soluble cellodextrins, mannodextrins and lactose to their corresponding lactones. In addition, cellulose, xylan and lignin can be degraded in the presence of H_2O_2 and chelated Fe ions by CDH [1–3]. The CDH is secreted out of the fungi, which were discovered in decayed wood. Fungi that secrete CDH are phytopathogens and have the ability to degrade wood. However, they must be able to penetrate the plant cell wall in order to infect plant cells.

Bioenergy is a promising alternative to fossil energy. Pretreatment of inedible biomass is a necessary process for bioconversion to sugar, which is the intermediate step in the process of producing products from biomass [4–7]. Biomass pretreatment is the key to low cost bioconversion of cellulosic biomass to sugar because of the rigid and hard-degradable structure of the cell wall. Actually, pretreatment is used to liberate cellulose from amorphous lignin and hemicellulose. Chemical pretreatment processes using acid and alkali reagents have been widely studied. Acid pretreatment hydrolyzes the structure of the cell wall, especially hemicelluloses, and the effectiveness of this treatment is dependent on the acid strength; however, only dilute acids should be used in order to prevent over-degradation of the biomass structure [8,9]. To achieve selective hydrolysis using dilute acid, the pretreatment reaction temperature must be around 150 °C [9]. Alkali pretreatment solubilizes the amorphous portion, mainly lignin, which is necessary for woody biomass pretreatment. During alkali pretreatment, salvation and saphonication takes place and this causes swelling of biomass [8,9]. Ammonia is a well known solvent for alkali pretreatment, and due to its environmental problems, this chemical must be recovered and recycled after use. Also, NaOH and KOH have also been widely studied because they are cheaper than ammonia [10,11].

Many studies on biological pretreatment have also been performed. But the effect is not strong and pretreatment requires a relatively long time. Thus, multiple pretreatment steps that are associated with other pretreatment processes must be studied [12].

In our previous work, CDH was successfully produced from *Phanerochaete chrysoporium* using rice straw as a carbon source

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[13]. Also, rice straw and barley straw were pretreated with dilute sulfuric acid and aqueous ammonia to enhance enzyme accessibility. In addition, the pretreatment conditions were optimized using statistical methods [14].

In this study, the rice straw after fermentation for CDH production was reutilized as a biomass, which can be converted into sugar. Changes in the composition of the rice straw after different chemical pretreatments, such as dilute acid pretreatment and aqueous ammonia pretreatment, were investigated. The goal of this study was to investigate changes in the composition of glucan and xylan during the degradation of rice straw by *P. chrysoporium*. In addition, to increase exposure of the substrate to the enzyme, aqueous ammonia was utilized to remove lignin, which might not be digested by microorganism. Furthermore, dilute acid pretreatment was utilized to increase the crystallinity index (CrI). X-ray diffractometry was used to measure CrI and to determine the molecule structure of pretreated rice straw.

2. Material and methods

2.1. Feedstock, enzymes and saccharification

Rice straw, which was used as a feedstock, was obtained from a Biochemical Engineering Laboratory in Kyong-gi University, Korea. The rice was stored at 20 °C and a relative humidity (RH) of 70% in the dark. Celluclast® and Novozyme188® were used for enzymatic hydrolysis of biomass. Celluclast® is a cellulose from *Trichoderma reesei* and Novozyme188® was obtained from *Aspergillus niger*. The Celluclast® and Novozyme188® activities were 60 FPU and 15 CBU, respectively. Enzymatic hydrolysis for enzyme digestibility was investigated according to the NREL standard procedure [15]. The reaction conditions were as follows; 50 °C, 0.1 M citrate buffer (pH 4.8) and 150 rpm.

2.2. Production of CDH by P. chrysoporium

Thirty milliliters of 0.2% Tween 80 solution was added to test tubes containing PDA media. After shaking, each flask was inoculated using 20 mL of a spore solution containing *P. chrysoporium* and incubated at 27 °C on a rotary shaker that was set at 150 rpm for 3 days. Seed culture was carried out in a 500 mL Erlenmeyer flask containing 200 mL of YM broth. The composition of the media was as follows (per liter): 2.28 g of $(NH_4)_2HPO_4$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.74 g of $CaCl_2$, 0.01 g of $FeCl_3$, 0.0158 g of $NaNO_3$, 6.6 mg of $ZnSO_4 \cdot 7H_2O$, 3.8 mg of $MnSO_4 \cdot H_2O$, 1 mg of $CoCl_2 \cdot 6H_2O$, 0.1 mg of thiamine-HCl, 6.75 g of succinic acid, and 10 g of microcrystalline cellulose. The seed culture broth (10%) was inoculated in a 500 mL Erlenmeyer flask containing 200 mL of main medium and incubated at 27 °C with shaking as described above for 12 days. Fermentation was performed in 5 L stirred tank reactor (STR) with an operation volume of 3 L using the medium optimized in the flask culture. The culture temperature was maintained at 27 °C. The impeller speed and aeration rate were maintained at 250 rpm and 1.5 vvm, respectively. Samples were taken regularly on a daily basis during the culture period. An antifoam agent was added when needed [16].

2.3. Pretreatment processes of biomass

Dilute acid pretreatment (DAP) of the rice straw was performed in an oil bath using a well-sealed tube reactor that had a diameter of 1.2 cm and length of 18 cm. The oil bath consisted of a pre-heated bath and cooling bath. The temperature of the pre-heated bath was maintained at 210 °C for fast heat transfer, whereas that of the cooling bath was kept at room temperature. Pretreatment with aqueous ammonia (SAA, Soaking in Aqueous Ammonia) was carried out at 60 °C, 250 rpm, 15% ammonia, 24 h, and at a solid–liquid ratio of 1:12. After pretreatment, the solids were separated by filtration and washed with distilled water to remove any remaining ammonia until the pH was neutral. The samples were then dried at 45 °C until the weight did not change.

2.4. Analytical methods

The absolute composition of the solid biomass was analyzed according to National Renewable Energy Laboratory (NREL, USA) standard procedures [15]. For dilute acid hydrolysis, the biomass mixture with a diluted acid solution of sulfuric acid (72%, w/w) was heated to 121 °C in an autoclave. After heating and cooling, the mixture was neutralized with calcium carbonate. The supernatant of the biomass composition was then analyzed by High-performance liquid chromatography (HPLC) using an Aminex® HPX-87 H ion exclusion column (BIO RAD). The HPLC conditions were as follows: a column temperature of 50 °C, a refractive index detector, 0.005 N H_2SO_4 mobile phase and flow rate of 0.8 mL/min. The amount of solubilized xylose produced by the DAP process and sugar recovery after saccharification were also measured by HPLC.

To measure the crystallinity index (CrI), XRD (Miniflex II, Rigaku, Japan) analysis was performed to produce spectra using the θ-2θ method [16,17]. The XRD was operated at 45 kV and 30 mA at room temperature. The anode material was copper (Cu) and the K-α (irradiation) was 1.544 Å. The scan range was 10–90° and the step size was 0.01°. The intensities of the amorphous ($2\theta = 18°$) and crystal regions ($2\theta = 22°$) as reported by Segal et al. [16] were utilized to calculate the CrI as follows:

$$\text{Crystallinity Index(CrI)} = \frac{I_{22^\circ} - I_{18^\circ}}{I_{22^\circ}} \times 100(\%)$$

where I_{θ° was the intensity at the corresponding θ.

3. Results and discussion

3.1. Investigation of rice straw after fermentation

Previously, we evaluated CDH production from different microbial strains [13]. Determination of strain and optimization of media composition and culture conditions was performed in a 5 L stirred tank reactor (STR). In this process, *P. chrysoporium* ATCC 32629 was used for efficient CDH production, and rice straw was utilized as the carbon source. *P. chrysoporium* is a white rot fungi that can be separated from rotten wood or grass [1,18]; thus, rice straw is a good carbon source for *P. chrysoporium*. CDH was produced by fermentation in a 5 L STR, solid/liquid separation was performed using vacuum filtration. The separated rice straw after fermentation (RF) was washed using distilled water (DW) and dried in an oven at 40 °C until no further change in the weight of the rice straw was observed. After drying, the dried rice straw had aggregated and hardened. The aggregated rice was then broken up and homogenized using a homogenizer. The prepared rice straw was weighed and analyzed. The sugar composition was analyzed following the standard procedure of NREL [15]. Table 1 shows the results of the composition analysis and initial reaction rate of the enzymatic hydrolysis reaction. The glucan and xylan content in the raw rice straw was 46.23% and 19.05%, respectively, and the remaining 34.68% consisted of other lignin containing sugars. After fermentation, the dry weight of the separated rice straw decreased to approximately 57.2% of the raw rice straw weight, which was fed into the 5 L STR. The rice straw after fermentation (RF) consisted of 28.77% glucan, 16.39% xylan and 54.81% others. The results of the compositional analysis show that *P. chrysoporium* consumed about